WP 2.2: Anti- and probiotics in preterm and term pigs

1. Related WPs, MG contact: Synergy with WP 1.3a,1.4b,1.5,1.6b,WP3. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
Anders D. Andersen, post doc, Clinical & Exp. Nutr., NEXS, Univ. Copenhagen, adan@life.ku.dk (10%)
Ann-Sofie Riis Poulsen, PhD stud, Dept. Animal Sci, Aarhus University, Ann-Sofie.RiisPoulsen@agrsci.dk (30%)
Axel Kornerup Hansen, Prof., Dept. Veterinary Disease Biology, Univ. Copenhagen, akh@sund.ku.dk (5%)
Charlotte Lauridsen, Ass. Prof., Microbiology/Immunology, Aarhus Univ., Charlotte.Lauridsen@agrsci.dk (5%)
Dennis Nielsen, Ass. Prof., Dept. Food Science, Univ. Copenhagen, dn@life.ku.dk (10%)
Gorm Greisen, Prof., Copenhagen University Hospital, Gorm.Greisen@regionh.dk (5%)
Hanne Frøkiær, Prof., Dept. Veterinary Disease Biology, Univ. Copenhagen, hafr@sund.ku.dk (5%)
Malene Cilieborg, post doc, Clinical & Experimental Nutr., NEXS, Univ. Copenhagen, macilie@life.ku.dk (5%)
Nuria Canibe, Ass. Prof. Aarhus University, Nuria.Canibe@agrsci.dk (5%)
Päivi S. Takkunen, PhD stud., Clinical & Experimental Nutrition, NEXS, paivi@life.ku.dk (50%)
Per Sangild, Prof., Clinical and Experimental Nutrition, NEXS, Univ. Copenhagen, psa@life.ku.dk (10%)
Preben Bodstrup Rasmussen, company, ARLA Foods amba, preben.bodstrup.rasmussen@arlafoods.com (5%)
Thomas Thymann, Ass. Prof., Clinical & Experimental Nutrition, NEXS, Univ. Copenhagen, ttn@life.ku.dk (20%)

3. Main aim and sub-aims:
The main aim is to assess the effect of probiotics supplementation on postnatal immunity, gut and brain development. In this context we want to assess if the effect is a) dose-dependent, b) dependent on timing of first inoculation, c) dependent on use of antibiotics, d) dependent on degree of maturity.

4. Background and a central hypothesis:
Postnatal gut colonization starts immediately after birth and is influenced by environmental bacteria. Probiotics supplementation may be a way to manipulate the early colonization to prevent gut disease. Addition of probiotics to milk feedings has been speculated to promote colonization of beneficial gut bacteria, suppress pathogens and stimulate immune development. However, the current level of evidence limits widespread use of probiotics, especially for vulnerable newborn infants as their potential positive effects are currently not predictable enough. We speculate that the unpredictable effects of probiotics are in part due to the highly variable use of neonatal antibiotics that may hamper the probiotic effect. Also the timing of probiotics inoculation after birth may influence how well they establish and display their beneficial effects. A major factor that influences the gut microbiota, is the use of antibiotics. Antibiotics are essential to prevent and treat infections, especially for the weakest newborn infants. Preventive antibiotics treatment around birth may prevent neonatal infections, but the effects on immune, gut and brain maturation are not well known. Both positive and negative effects have been demonstrated. The interaction between antibiotics and probiotics in newborn (preterm and term) infants remains unknown despite its potential high importance for clinical outcome for infants. *We hypothesize that antibiotics will initially benefit the immature immunity, gut, and brain via the reduced bacterial load, and that subsequent probiotics used at the optimal time, dose and strain combination will improve maturation.*

5. Key analyses and methods:
In experiment 1 preterm piglets are derived by cesarean section and reared in incubators. All pigs are provided with total parenteral nutrition for 2-3 days via the umbilical artery. After this they are gradually transitioned to full enteral nutrition with milk formula. Five different doses of a selected probiotic will be given daily starting immediately after birth. In experiment 2 we take the optimal dose from exp. 1 and inoculate preterm piglets either immediately after birth, or 3 days after birth. Half of the pigs from each group will be exposed to antibiotics treatment (e.g. Ampicillin, Gentamycin, Metronidazol and to a less extent Vancomycin) (2X2 factorial design). The study duration will be up to 12days depending on their clinical status. Endpoints include

clinical assessment, growth velocity, diarrhea scoring and NEC symptoms. Tissue samples and samples from the gut luminal content are collected and analyzed (proinflammatory cytokines, gene expression, mucosal digestive function, microbial composition, SCFA). Brain endpoints include open field test and BBB.
In Experiment 4, probiotics intervention is studied in term piglets in collaboration with Aarhus University, Foulum. Half of the piglets in 2 litters of term piglets are inoculated with probiotics either immediately after birth or 3 days after birth. Clinical and laboratory endpoints as mentioned above.

6. Expected results:
We expect to determine how profound manipulation of the gastrointestinal microbiota during early postnatal colonization can impact on gut and brain development. Secondly we expect to determine any dose-response effect seen after administration of various levels of probiotic administration. We expect to determine the optimum level and optimal timing under the given experimental conditions.
Predicted publications:
1) Optimal dosing and timing of probiotics supplementation in early life.
2) Antimicrobial effects in early life and subsequent recolonization with selected probiotic strains.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol			x																	
Sample collection				x	x															
Clinical/ behaviour				x	x															
Growth				x	x															
Cytokines						x	x													
Gene expression						x	x													
Mucosal digestive func						x	x													
Microbial comp						x	x													
BBB						x	x													
Publication(s)								x	x	x	x									

8. Estimated budget from NEOMUNE:
2.5 mio DKK, derived from the KU-SCIENCE NEOMUNE budget (Thomas Thymann). 500,000 DKK, derived from the AU NEOMUNE budget (Charlotte Lauridsen).

9. Estimated budget from elsewhere:
0.5 mio DKK is achieved by self-financing (i.e. university funded salaries) and collaboration with antibiotics and probiotics partners.

10. Additional comments:
- This WP relates closely to WP2.1 and WP3 on the experimental side. All these WPs represent profound experimental manipulation of the gastrointestinal flora just after birth. Relative to WP2.1 (germ free conditions), WP2.2 represents a more clinically relevant model. All the animal studies are meant to rely on clinically relevant situations for infants (e.g. WPs 1.2b,1.4b,1.5) and the possible interventions for infants (WPs 1.3a,1.6b).
- It will be a challenge to choose the optimal product(s), timing and doses of both antibiotics and probiotics. Clearly it is impossible to test all clinically-relevant combinations. We will focus on products, timing of administration and doses that are currently used in the neonatal clinics around the world. The results from WP 1.4b (data base work) will help to determine this.